Spore Retention and Release from Overwintering Fern Fronds

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The need for ecological data on ferns is becoming increasingly apparent as more effort is directed toward an understanding of the significance of their morphological and physiological diversity (Wagner, 1973). If detailed ecological studies of fern sporophytes have to date been too few, such studies on the gametophyte generation are almost non-existent. The reproductive cycle of ferns has been known for over a century, and more than a thousand articles on fern gametophytes have been published, half of these in the last quarter century (Miller, 1968; Näf, 1975; Nayar & Kaur, 1971). However, nearly all data on gametophyte growth and sexual reproduction have been based on laboratory observations. Several factors have contributed to this paucity of information on gametophyte ecology, but perhaps the most important has been a widely held notion that gametophytes cannot be found in nature, or if found, cannot be identified. Several recent studies have indicated to the contrary, that *in situ* gametophyte studies not only are feasible, but that they are essential for the integration of existing laboratory data into studies on the natural history of ferns (Cousens, 1973; Holbrook-Walker & Lloyd, 1973; Lloyd, 1974; Farrar & Gooch, 1975).

To investigate further the feasibility of studying fern gametophytes in nature, we have begun a long-term observational study of fern reproduction in Woodman Hollow, a relatively isolated canyon in central Iowa, in which 13 species and 11 genera of ferns occur (see *Table 1*). This study is designed to answer the following questions. When are spores available for germination? When and where does reproduction occur and how is it influenced by micro- and macroclimates? When and by what breeding systems are sporophytes produced? Does sexual reproduction occur in nature on a regular basis for all species? Results of the first year of study (Farrar & Gooch, 1975) indicate that the data needed to answer these and other questions will be forthcoming. Here we report some unexpected data relevant to the question of when spores are available for germination.

Observations made on the time of spore maturation and first release during the growing season gave results which were similar to those of Hill and Wagner (1974) for pteridophytes in Michigan. Differences found in the two studies were no greater than might be expected due to differences in latitude, climate, habitat, and seasonal variation. Our observations also support their estimate that most spores of a given species are released during a period of about two weeks. However, a two week period of maximum release, if taken as a guide to the duration of spore release, may be very misleading. Our observations at Woodman Hollow indicate that for most species, significant quantities of spores are retained on the fronds after the initial release period and may be dispersed during a much longer period.

Only in *Botrychium virginianum* and *Osmunda claytoniana* were essentially all

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of the spores shed in a period as short as two weeks after spore maturation. These species have large, smooth-walled sporangia which mature simultaneously. Furthermore, these species have dimorphic pinnae, and the fertile pinnae wither and often disappear soon after maturation.

In the remainder of the species in our study, all sporangia in a sorus do not mature simultaneously. This differential maturation in itself lengthens the period of spore release, but an additional effect of this mixed sorus condition is that late-maturing sporangia are frequently covered by older, dehisced sporangia. As a result, they may be physically unable to dehisce and shed their spores normally, and often are prevented from opening at all. Thus, the sorus may remain indefinitely with a mixture of opened sporangia containing no spores (those which opened first), opened sporangia which still contain some or all of their spores, and unopened sporangia containing their full complement of spores.

Spore retention on the fern fronds varies among species and is dependent upon several factors. These include the number of sporangia per sorus, the presence of hairs or an indusium over the sorus, the type and degree of persistence of the indusium, and the time of maturation of the fertile frond. We observed a number of species which, after an initial flush of fertile fronds, continued to produce additional fertile fronds for the remainder of the growing season. Fronds maturing late in the season, especially of *Adiantum pedatum*, *Asplenium rhizophyllum*, and *Polypodium virginianum*, often had large numbers, occasionally approaching 100%, of unopened sporangia.

To quantify as much as possible this extended period of spore retention and release, fertile fronds of species in the study area were collected in December and again in March. The fronds were examined under a dissecting microscope and an estimate was made of the percent of unopened sporangia and the total number of spores remaining on the frond. Because of the generally deteriorated condition of the fronds, precise counts of sori, sporangia, and free spores could be made only with considerable difficulty. Thus, the spore estimates were limited to the orders of magnitude listed in *Table 1*. Ten or more sori were examined on each frond. The estimates of spores per frond were based on the number of unopened sporangia, the number of spores which could be removed from the dried frond, and the number of spores which could be seen remaining on the frond. Taking into account the inherent problems with the methodology of the spore assay and assuming that the estimates are not in error by more than an order of magnitude, it was obvious that for most species, large numbers of spores were retained on the fronds throughout the winter.

Spore viability was also tested for each collection by sowing the spores on mineral nutrient agar and measuring percent germination after three weeks. Germination in December ranged from 49% to 96%. Differences in germination percentages obtained in December and March probably represent variation between plants of the same species, since the overall range in March was similar (48–88%) to that in December and the number of species showing an increase was nearly as great as those showing a decrease in germination (*Table 1*). The change in *Cystopteris bulbifera* was most dramatic and may represent a real decrease in spore

viability for this species. To determine whether unopened sporangia of late-maturing fronds contained mature and viable spores, fronds of *Asplenium rhizophyllum* were divided into three lots on the basis of the number of unopened sporangia. These lots, when tested independently, showed similar germination percentages, indicating that indehiscence of sporangia, at least in this species, was not due to spore immaturity.

From December to March, a definite decrease in the number of unopened sporangia occurred only in late-maturing fronds of *Asplenium rhizophyllum*, which in December had greater than 90% unopened sporangia, and in *Matteuccia struthiopteris*, which was observed to be releasing spores both in December and in March. The total number of spores per frond appears generally to have remained relatively unchanged; however, our method of analysis may not have been sufficiently sensitive to detect the changes that did occur. That some spores were released from December to March is indicated by the decrease in unopened sporangia in *Asplenium rhizophyllum* and *Matteuccia struthiopteris* and by a measurable decrease in numbers of spores per frond in *Adiantum pedatum*, *Cystopteris bulbifera*, *Dryopteris goldiana*, and *Woodsia obtusa*.

TABLE 1. SPORE PRESENCE AND VIABILITY ON FERN FRONDS COLLECTED IN DECEMBER AND MARCH IN WOODMAN HOLLOW, IOWA

	No. fronds analyzed		*% sporangia unopened*		*No. spores/frond (× 1000)*		*% spore germination (number counted)*	
	Dec.	*Mar.*	*Dec.*	*Mar.*	*Dec.*	*Mar.*	*Dec.*	*Mar.*
Adiantum pedatum	6	3	1-10	same	10-100	1-10	77 (416)	67 (213)
Asplenium rhizophyllum	35	3	1-10	same	1-10	same	81 (237)	87 (378)
Asplenium rhizophyllum	6	—	10-20	—	10-100	—	84 (210)	—
Asplenium rhizophyllum	9	2	>90	>75	100-1,000	same	81 (308)	—
Athyrium filix-femina	3	4	1-10	same	10-100	same	49 (645)	73 (541)
Cryptogramma stelleri	0	2	—	>75	—	10-100	—	63 (183)
Cystopteris bulbifera	4	4	<1	same	1-10	0.1-1	62 (444)	6 (226)
Cystopteris fragilis	5	4	<1	same	1-10	same	68 (190)	58 (248)
Dryopteris goldiana	4	4	1-10	<1	10-100	1-10	50 (231)	48 (306)
Dryopteris spinulosa	4	4	<1	1-10	10-100	same	52 (307)	84 (703)
Matteuccia struthiopteris	3	1	>90	>80	100-1,000	same	96 (599)	95 (668)
Polypodium virginianum	3	5	10-50	same	100-1,000	same	70 (224)	88 (258)
Woodsia obtusa	6	4	1-10	same	10-100	1-10	82 (403)	59 (230)
Botrychium virginianum	no fertile fronds found							
Osmunda claytoniana	no fertile fronds found							

The data thus indicate that for most of the fern species in Woodman Hollow, sporophyte fronds of the previous year retain large numbers of spores throughout the winter and into the growing season of the following spring. Furthermore, it appears that some spores continue to be shed from these fronds as the winter progresses. The fate of the spores that are shed, or of those that remain on the fronds, has yet to be determined. The old, spore-bearing fronds are generally flattened against the substratum by early spring, and further release of their spores into air currents must be greatly reduced. Nevertheless, some spores could certainly germinate in the vicinity of the sporophyte fronds if a suitable habitat were available.

As yet, we have detected establishment of large numbers of gametophytes of only three species, *Adiantum pedatum, Cystopteris fragilis*, and *Woodsia obtusa*, and this has occurred in the fall. Our failure to observe gametophyte establishment of other species, or of these species at other times of the year, may be due to unfavorable weather conditions, or may reflect the inability of some species to reproduce regularly or extensively through the production of gametophytes. It most certainly reflects the rudimentary state of our knowledge of fern reproduction in nature. It may well be true that significant gametophyte establishment results only from spores released during the sporophyte growing season. However, until this is proven, workers studying gametophyte ecology in temperate areas must consider the possibility that significant reproduction may also result from spores shed from overwintering fronds.

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